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Performance and Meat Quality of Broiler Chicken with the Addition of Guanidino Acetic Acid and Betaine as Feed Additive

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Guanidino acetic acid is a precursor of creatine which is synthesized from the amino acids glycine and arginine by transferring the amidino group from arginine to glycine, catalyzed by the enzyme L-arginine: glycine amidine transferase (AGAT) which takes place in the kidneys and pancreas, while betaine is known as a group donor methyl which functions in many metabolic processes such as energy metabolism, protein synthesis, carnitine and creatine. This research aims to determine the effect of adding Guanidino Acetic Acid (GAA) and betaine as feed additives in improving the performance and meat quality of broiler chickens. This research used 100 broiler chickens (DOC) with 6 chickens per unit which were reared for 35 days. The experimental design used was a Completely Randomized Design (CRD) with 5 treatments and 4 replications. Treatment consisted of P0 (Commercial feed,) P1 (Commercial feed + 0.06% GAA and no Betaine), P2 (Commercial feed + 0.06% GAA + 0.01% Betaine), P3 (Commercial feed + 0.12 % GAA and no Betaine), P4 (Commercial ration + 0.12% GAA + 0.01% Betaine). The parameters observed were performance, commercial carcass and muscle histomorphology. The results of the study showed that the addition of GAA and betaine had a real effect ($P<0.05$) and improved the performance of commercial carcasses and showed a better response to muscle histomorphology. The conclusion was that the addition of GAA 0.12% and betaine 0.01% through feed could improve the performance, commercial carcasses and muscle histomorphology of broiler chickens.

Keywords: Broiler chicken, Betaine, Guanidino Acetic Acid, Betaine, Creatine, Broiler chickens,Feed additives, Performance, Meat quality.

INTRODUCTION

Broiler chickens are a type of breed of chicken that is raised with the aim of producing meat as a source of animal protein (Bulkaini *et al.* 2021). To achieve very fast growth and high productivity, feed is needed that contains adequate nutrients (Ravindran, 2013; Muhammad *et al.* 2023). According to (Dobenecker and Braun, 2015), The main raw material composition of broiler chicken feed produced is corn and donkey meal. As a result, feed produced by manufacturers has a low creatine content (Hardiyanto, 2022). So it has not been able to support the speed of growth of broiler chickens, especially in terms of weight gain without fat. Creatine is a component that supplies phosphate groups to adenosine diphosphate and recycles it into adenosine triphosphate (ATP) which can be used for maintenance and growth (Boney *et al.* 2019). When exposed to low pH, humidity and high temperatures, creatine can degrade into creatinine and is not useful for animals (Dobenecker and Braun, 2015). So creatine is not effective as a feed additive or applied directly, because of its instability and high price. Therefore, it is necessary to look for alternative materials that are chemically stable and cheaper. One type of alternative ingredient is *Guanidino acetic acid* (GAA) because it is cheaper and more chemically stable and is easily converted into creatine by the body (Ren *et al.* 2019).

Guanidino acetic acid or guanidino acetic acid or also called glycoamine is a precursor of creatine (Portocarero *et al.* 2021). Guanidino acetic acid is synthesized from the amino acids glycine and arginine by transferring the amidino group from arginine to glycine, catalyzed by the enzyme L-arginine: glycine amidi notransferase (AGAT) and takes place in the kidneys and pancreas (Ostojic, 2015). However, exogenous GAA supplementation does not go through a long process, so the body's biochemical processes become more efficient.

Supplementation with high levels of GAA and insufficient methyl donors to convert GAA into creatine prevents biosynthesis from being maximized (Sharma *et al.,* 2022).

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The methyl group donated by S-adenosyl methionine to GAA comes from methionine. So, a methyl donor is needed that is able to maximize creatine synthesis without having negative effects. Betaine is known as a methyl group donor which functions in many metabolic processes such as energy metabolism, protein synthesis, carnitine and creatine (Park and Kim, 2017). Betaine converts homocysteine back to methionine, and after donating its methyl group, it is converted to glycine (Sharma *et al.* 2022). He *et al.* (2015), reported that betaine can increase feed consumption, body weight gain, and feed conversion in broiler chickens. So, this research aims to determine the effect of the combination of GAA and betaine on performance (feed consumption, body weight gain and feed conversion ratio), commercial carcass and muscle histomorphology of broiler chickens.

MATERIALS AND METHODS

The research was carried out from July to August 2023, at the Poultry Production Laboratory, Faculty of Animal Husbandry, Hasanuddin University, South Sulawesi, Indonesia. A week before DOC is put into the cage, sanitation and disinfection of the cage is first carried out to kill and break the chain of development of microorganisms. This study used the Lohmann chicken strain (MB202) as many as 100 broiler chickens divided randomly into 20 units for five treatments and four replications. First of all, broiler chickens are reared through a brooding period of 100 aged chicks 1 day for 7 days. Broiler chickens were reared on day 8 to day 35. The treatment group consisted of P0 (Commercial Feed as control); P1 (Commercial ration + 0.06 % GAA and no Betaine); P2 (Commercial ration + 0.06 % GAA + 0.01% Betaine); P3 (Commercial ration + 0.12% GAA and no Betaine); and P4 (Commercial ration + 0.12% GAA + 0.01% Betaine). During maintenance, food and drinking water are provided ad libitum based on treatment.

Table 1. Nutrient Composition in experimental diet.

Nutritional Substances	**Composition (%)**	
	Phase *Starter*	**Phase *Finisher***
Water content (maks)	12.00	12.00
Crude protein (min)	20.00	19.00
Crude fat (min)	5.00	5.00
Crude fiber (maks)	5.00	6.00
Ash (maks)	8.00	8.00
Calcium (min)	0.60	0.55
Phosphor (min)	0.50	0.45
Amino acid		
Lysine (min)	1.20	1.05
Methionine (min)	0.45	0.40
Methionine + Cystine (min)	0.80	0.75
Tryptophan (min)	0.19	0.18
Threonine (min)	0.75	0.65

Analysis results from the manufacture

The parameters measured in this study were performance, commercial carcass and muscle histomorphology of broiler chickens. Broiler chicken performance consists of feed consumption which is obtained from the amount of feed consumed minus the remaining feed given and divided by the number of chickens. body weight gain is obtained from the difference in chicken body weight and feed conversion is obtained from the amount of feed consumed divided by body weight gain. Commercial carcasses are obtained by slaughtering them, then removing the feathers, removing the digestive organs and separating each part of the commercial carcass. Such as the breast, wings, back and legs which will be weighed to get a percentage of the commercial carcass weight. Then muscle histomorphological testing uses breast and leg muscles that have been separated from bones, organs and other muscles.

***Ethical regulation*:** The rearing management in this research refers to the Australian Animal Welfare Standards and Guidelines for Poultry and the slaughter of broiler chickens in this research refers to the Indonesian National Standard (SNI) number 99002 of 2016 concerning halal poultry slaughter.

***Statistical analysis*:** The research design was carried out using a Completely Randomized Design (CRD) with a significance level of 5%. If the data is significantly different at the P<0.05 on the measured parameters, then the differences between treatments were analyzed by an orthogonal contrast test.

RESULTS AND DISCUSSION

***Feed consumption*:** The result in Table 2 shows that the addition of GAA and betaine as feed additives on the performance of broiler chickens has a significant effect (P<0.05) on feed consumption. The feed consumption of broiler chickens in treatment P4 (commercial ration + 0.12% GAA + 0.01% Betaine) was significantly higher, namely 2226.30 ± 52.57 grams/head compared to other treatments. Further tests showed that feed consumption in Group I (P0 Vs P1; P2; P3; P4) was significantly different (P<0.05), this was because P0 feed did not contain GAA and betaine. GAA functions to maintain intestinal morphometrics in chickens and betaine functions to maintain intestinal health during osmotic disturbances, so that it can increase feed consumption in broiler chickens. This is in line with the opinion of Ahmadipour *et al.* (2018) who said that there is an effect of GAA supplementation in feed which causes better development of the small intestine, especially greater height and width of the intestinal villi. Furthermore, He *et al.* (2015), reported that betaine can increase feed consumption in broiler chickens by exerting morphological changes in the intestinal epithelium during osmotic disturbances caused by heat stress, thereby leading to increased feed efficiency and growth rate.

Table 2. Performance of broiler chickens with the addition of GAA and Betaine as feed additives in the ration.

Parameter	**Treatment**				
	P0	**P1**	**P2**	**P3**	**P4**
Feed consumption (g/bird)	1985.20 ± 51.27	2091.20 ± 79.75	2212.75 ± 64.77	2127.10 ± 62.77	2226.30 ± 52.57
Weight gain (g/bird)	1348.14 ± 205.32	1476.05 ± 150.68	1903.33 ± 101.78	1583.30 ± 184.38	2022.60 ± 249.69
FCR	1.55 ± 0.21	1.43 ± 0.13	1.16 ± 0.07	1.31 ± 0.11	1.11 ± 0.13

P0 (Commercial Feed as control); P1 (Commercial ration + 0.06 % GAA and no Betaine); P2 (Commercial ration + 0.06 % GAA + 0.01% Betaine); P3 (Commercial ration + 0.12% GAA and no Betaine); and P4 (Commercial ration + 0.12% GAA + 0.01% Betaine).

Table 3. Performance of broiler chickens (orthogonal contrast test).

Parameter	**Contrast**	**Significant**
Feed consumption	P0 Vs P1; P2; P3; P4	0.01*
	P1; P3 Vs P2; P4	0.00*
	P1 Vs P3	0.43^{tn}
	P2 Vs P4	0.76^{tn}
Weight gain	P0 Vs P1; P2; P3; P4	0.00*
	P1; P3 Vs P2; P4	0.00*
	P1 Vs P3	0.42^{tn}
	P2 Vs P4	0.37^{tn}
Feed conversion ratio	P0 Vs P1; P2; P3; P4	0.00*
	P1; P3 Vs P2; P4	0.00*
	P1 Vs P3	0.24^{tn}
	P2 Vs P4	0.59^{tn}

*significant. ^{ns} non significant

Further tests for Group II (P1; P3 Vs P2; P4) showed significantly different results (P<0.05), the difference was possibly due to the betaine content in treatments P2 and P4. Betaine mixed into feed can increase the regulation of gene expression in intestinal epithelial cells. Alhotan *et al.* (2021) reported that betaine supplementation can regulate the formation of cytokines and upregulate gene expression that helps maintain intestinal epithelial cells thereby limiting performance decline during heat stress. Meanwhile, further tests on Group III (P3 Vs P4) and Group IV (P2 Vs P4) showed that they were not significantly different (P> 0.05), this is probably due to the GAA concentration level in the feed not having a significant influence on feed consumption in Group III (P3 Vs P4) and Group IV (P2 Vs P4) with the addition of betaine. The results of this study are in accordance with Zhang *et al.* (2021) who stated that there was no difference in body weight gain and feed consumption when supplemented with GAA 600 and 1,200 mg/kg.

***Weight Gain*:** The increase in body weight of broiler chickens is influenced by the type of feed given. The addition of GAA and betaine as feed additives had a significant effect (P<0.05) on the increase in body weight of broiler chickens. The results of Table 2 show that body weight gain in the P4 treatment (commercial ration + 0.12% GAA + 0.01% Betaine) was significantly higher, namely 2022.60 ± 249.69 grams/head compared to other treatments. This is in line with the high consumption of P4 feed of 2226.30 ± 52.57 grams/head obtained during the process of rearing broiler chickens. The increase in body weight of chickens is closely related to the consumption of feed provided. The more feed a chicken consumes, the higher its body weight gain will be. This is in line with the opinion of Listyasari *et al.* (2022) which states that the increase in body weight of broiler chickens is directly proportional to feed consumption, the higher the increase in body weight of the chicken, the higher the feed consumption. Further tests showed that the increase in body weight of broiler chickens in Group I (P0 Vs P1; P2; P3; P4) was significantly different (P<0.05). This is because the presence of GAA in feed is able to meet energy needs for metabolic processes and tissue growth. Mousavi *et. al.*, (2013) concluded in his research that the addition of GAA can increase energy efficiency in broiler chickens at higher energy levels, thereby utilizing energy for growth, in this case weight gain (Sidadolog and Yuwanta, 2009). Furthermore, the betaine content contained in feed functions to regulate myogenic processes and growth. This is in line with Chen *et al.* (2018) who stated that betaine regulates myogenic gene expression and the insulin-like growth factor-1 signaling pathway to increase muscle protein deposition for body weight gain. Then in II (P1; P3 Vs P2; P4) showed significantly different results (P<0.05) due to the betaine content in treatments P2 and P4. This is because betaine functions as a methyl donor in transmethylation reactions to form important substances in increasing body weight gain. This is in line with Sharma *et al.* (2022) who stated that betaine is a good supplement because betaine converts

Table 4. Commercial broiler chicken carcasses with the addition of GAA and Betaine as feed additives.

Parameter	Treatment				
	P0	**P1**	**P2**	**P3**	**P4**
Leg (%)	31.25 ± 0.42	31.24 ± 0.56	31.58 ± 1.03	31.37 ± 1.10	31.75 ± 1.42
Wing (%)	12.29 ± 0.11	11.90 ± 0.58	11.33 ± 0.60	11.87 ± 0.26	10.71 ± 0.33
Back (%)	25.26 ± 0.27	25.73 ± 1.01	24.93 ± 0.40	25.73 ± 0.77	24.85 ± 1.79
Breast (%)	30.96 ± 0.44	31.00 ± 0.41	32.08 ± 1.29	32.02 ± 1.22	33.40 ± 0.56

P0 (Commercial Feed as control); P1 (Commercial ration + 0.06 % GAA and no Betaine); P2 (Commercial ration + 0.06 % GAA + 0.01% Betaine); P3 (Commercial ration + 0.12% GAA and no Betaine); and P4 (Commercial ration + 0.12% GAA + 0.01% Betaine).

Table 5. Commercial cascass of broiler chickens (orthogonal contrast test)

Parameter	Contrast	Significant
Wing	P0 Vs P1; P2; P3; P4	0.00*
	P1; P3 Vs P2; P4	0.00*
	P1 Vs P3	0.92^{tn}
	P2 Vs P4	0.05*
Breast	P0 Vs P1; P2; P3; P4	0.03*
	P1; P3 Vs P2; P4	0.01*
	P1 Vs P3	0.11^{tn}
	P2 Vs P4	0.04*

*significant. ^{ns} non-significant

homocysteine back into methionine, and after donating its methyl group, it will be converted to glycine. Putra *et al.* (2021) added that betaine can increase chickens' appetite when under heat stress, resulting in high weight gain.

Group III (P3 Vs P4) and Group IV (P2 Vs P4) showed that the results were not significantly different (P>0.05), this was because there was no influence on feed consumption in the further tests for Group III (P3 Vs P4) and Group IV (P2 Vs P4) which is probably caused by the level of GAA concentration in the feed does not have a significant effect. The results of this study are in accordance with Majdeddin *et al.* (2018) who observed that there was no significant increase in the increase in body weight of chickens with GAA supplementation at a concentration of 0.12%.

***Feed Conversion Ratio*:** Feed conversion or Feed Conversion Ratio (FCR) is a measure that can be used to assess the efficiency of feed use by calculating the ratio between the amount of feed consumed and body weight gain. The addition of GAA and betaine as feed additives had a significant effect (P<0.05) on feed conversion for broiler chickens. The results of Table 2 show that feed conversion in treatment P4 (commercial ration + 0.12% GAA + 0.01% Betaine) was significantly lower, namely 1.11 ± 0.13 compared to other treatments. This means that the P4 feed is more efficient for broiler chickens to consume feed to produce increased body weight or meat. A low feed conversion value indicates better feed use efficiency. This is in accordance with the opinion of Majid *et al.* (2022) who say that the feed conversion value can be expressed as a measure of feed efficiency which describes the level of livestock's ability to convert feed into a certain amount of production in certain units, especially meat production for broiler chickens.

Further tests showed that the feed conversion of broiler chickens in Group I (P0 Vs P1; P2; P3; P4) was significantly different (P<0.05). This is because the presence of GAA in the feed makes broiler chickens more efficient in consuming feed to produce body weight gain. The results of this study are comparable to research by Amiri *et al.* (2019) which reported that feed supplemented with GAA linearly reduced feed conversion in broiler chickens during maintenance. Indirectly, feed supplemented with GAA can reduce feed conversion.

Further tests showed that feed conversion for broiler chickens in Group II (P1; P3 Vs P2; P4) resulted in significantly different results (P<0.05). This is due to the addition of betaine to the feed which has a significant effect in reducing FCR. The results of this study are comparable to research by Chen *et al.* (2022) which reported that feed supplemented with 1,000 mg/kg betaine significantly reduced FCR in broiler chickens. The addition of betaine to feed causes decreased FCR in broiler chickens, which is related to intestinal health. This is in accordance with the opinion of Wen *et al.* (2021) that betaine can increase the growth of broiler chickens during the early growth period and increase feed efficiency which functions to maintain intestinal health to increase nutrient digestibility. Meanwhile, in further tests, Group III (P1 Vs P3) and Group IV (P2 Vs P4) showed that the results were not significantly different (P> 0.05). This was because there was no effect on feed consumption and body weight gain in the Group III (P1 Vs P3) and Group IV (P2 Vs P4) follow-up tests.

Leg: The addition of GAA and betaine as feed additives did not have a significant effect (P>0.05) on the leg weight of broiler chickens. The leg weight values did not differ much between all treatments, however the leg weight in treatment P4 tended to be higher by 31.75%. This result is higher than the leg weight value of Putra *et al.* (2021) which was maintained for 35 days, namely around 27.00%-29.88% by giving fermented lamtoro leaf flour and Semjon *et al.* (2020) with weight relative legs ranged from 26.20%-27.70% with fermented wheat bran supplemented with agrimony extract. This is because the protein consumption was 2522.31 ± 60.45 and the protein efficiency ratio was 0.80 ± 0.08 in treatment P4, which was higher than in other treatments, so it was able to produce a higher leg weight. This is in accordance with Mait *et al.* (2019) that the leg is the part of the carcass that produces the second most meat after the breast, so its development is influenced by the protein content in the feed.

The leg bones are used more for activities, so their growth and proportions follow body growth. This is in accordance with Tiya *et al.* (2022) that the leg in broiler chickens is a part of the body that is often used for activities, so the proportion of growth follows body growth so that it remains balanced. Activities that are used more on the leg muscles cause glycogen deposits to be used as a source of energy. This is in accordance with Septinova *et al.* (2018) that the leg muscles move more so that a large amount of glycogen stores are used as energy for movement activities. As a result, the amount of glycogen becomes less.

Wing: The addition of GAA and betaine as feed additives had a significant effect (P<0.05) on the wing weight of broiler chickens. The results of Table 4 show that the average wing weight ranges from 10.71% -12.29%. The research results showed that the highest P0 (Control) treatment was 12.29% and the lowest was P4 treatment, namely 10.71%. This wing weight is the same as research by Antarani *et al*. (2020) with the best results, namely around 10.38% -12.68% when maintained for 35 days. Further tests showed that the wing weights of broiler chickens in Group I (P0 Vs P1; P2; P3; P4), obtained significantly different results (P<0.05). The addition of GAA and betaine in feed with the aim of increasing wing weight is thought to be mostly focused on building breast and thigh muscles. This is because wing growth is also influenced by the large percentage of bone. This is in accordance with Megawati *et al.* (2020), who said that bone growth can influence body length and body weight. Ulupi *et al*. (2018), added that the height and low weight of the wings is also based on bone growth. The higher the wing bone weight, the higher the wing weight and vice versa, the lower the wing bone weight, the lower the wing weight.

Further tests in Group II (P1; P3 Vs P2; P4) and Group IV (P2 Vs P4) showed significantly different results (P<0.05), although statistically the addition of GAA and betaine was still less than optimal. However, the addition of betaine can have a significant effect compared to treatment without betaine. This is in line with Sinaga *et al*. (2020) that the availability of methyl from betaine donors allows the formation of amino acids as needed for muscle formation. So Group III (P1 Vs P3) with different GAA concentrations and without using betaine, the results were not significantly different (P>0.05), this was because there was no betaine content in the feed as a methyl donor which caused the weight of the wings to contain more bone.

Back: The addition of GAA and betaine as feed additives did not have a significant effect (P>0.05) on the back weight of broiler chickens. This is because the back is the part that is dominated by bones and has less potential to produce meat. This is in line with Anwar *et al* (2019) that the back is the part that has the highest proportion of bones compared to other

Table 6. Number of breast muscle myofibrils in broiler chickens

Parameter	Treatment				
	P0	**P1**	**P2**	**P3**	**P4**
Number of Myofibrils	147.83 ± 44.48	188.83 ± 75.70	240.92 ± 33.06	205.75 ± 55.39	286.33 ± 13.14
Myofibrils Diameter	30.09 ± 6.06	41.48 ± 8.74	52.49 ± 4.48	46.88 ± 2.72	54.47 ± 5.70

P0 (Commercial Feed as control); P1 (Commercial ration + 0.06 % GAA and no Betaine); P2 (Commercial ration + 0.06 % GAA + 0.01% Betaine); P3 (Commercial ration + 0.12% GAA and no Betaine); and P4 (Commercial ration + 0.12% GAA + 0.01% Betaine).

Table 7. Breast muscle histology (orthogonal contrast test)

Parameter	Contrast	Significant
Number of Myofibrils	P0 Vs P1; P2; P3; P4	0.00*
	P1; P3 Vs P2; P4	0.01*
	P1 Vs P3	0.63^{tn}
	P2 Vs P4	0.21^{tn}
Myofibrils Diameter	P0 Vs P1; P2; P3; P4	0.00*
	P1; P3 Vs P2; P4	0.00*
	P1 Vs P3	0.21^{tn}
	P2 Vs P4	0.64^{tn}

*significant. ^{ns} non significant

parts. Mait *et al*. (2019) added that the weight of the back is composed of mostly bone and a little muscle tissue.

The results of this study were higher than the back weight values of Mait *et al*. (2019) which ranged from 18.50 – 19.67% of the carcass percentage. This is due to the provision of GAA in the feed, while GAA is a precursor of creatine which acts as an energy reserve for bone growth and development. This is in accordance with research by Khajali *et al* (2020) that the growth and development of bones requires a large amount of energy to maintain, repair and reproduce. GAA of 0.6 g/kg has been shown to increase the tibial density of broiler chickens up to 24 days of age. In addition, the findings of a study by Zhao *et al*. (2019) that used betaine in feed revealed that increasing the size of pig muscle fibers increased skeletal muscle growth.

***Breast*:** Breast weight is the part of the carcass that produces the most meat and is liked by the public. The addition of GAA and betaine as feed additives had a significant effect (P<0.05) on the breast weight of broiler chickens. The results of Table 7 show that the breast weight in treatment P4 (commercial ration + 0.12% GAA + 0.01% Betaine) was significantly higher, namely 33.40% compared to other treatments. This result was due to the protein consumption and protein efficiency ratio in treatment P4 being higher compared to other treatments, so that the formation of breast muscle tissue was more optimal. This is in accordance with Anwar *et al*. (2019) that the breast piece is the part that contains the most muscle tissue. Muscle growth is influenced by protein, especially amino acids.

Further tests showed that the breast weight of broiler chickens in Group I (P0 Vs P1; P2; P3; P4), the results were significantly different (P<0.05). This is because the concentration of phosphocreatine in breast muscle cells has increased due to the addition of GAA in the feed. This is in accordance with Boney *et al*.(2019) that GAA in broiler chicken feed can influence the concentration of phosphocreatine in breast muscle cells which results in an increase in breast weight which is seen in the main effect of GAA inclusion. Majdeddin *et al*. (2018) added that apart from improving performance, the addition of GAA to broiler chicken feed was reported to increase the creatine content in the breast muscle. Meanwhile, further tests for Group II (P1; P3 Vs P2; P4) and Group IV (P2 Vs P4) with the addition of betaine showed significantly different results (P<0.05), adding betaine to the feed helped increase muscle mass in broiler chickens. This is in accordance with Cholewa *et al*. (2014) that the accumulation of betaine in cells increases sarcoplasmic osmolality, which helps increase muscle mass.

Group III (P1 Vs P3) with different GAA concentrations and without using betaine did not show significantly different results (P<0.05). This is thought to be due to the absence of betaine content in the feed which results in the availability of methyl group donor acceptors not being balanced by the availability of methyl group donor supplementation so that it does not function optimally. Asiriwardhana and Bertolo, (2022), said that adding GAA to feed will proportionally increase the consumption of methyl groups from SAM to produce more creatine. Thus, low availability of methyl groups can interfere with other important methylation reactions.

***Number of Myofibrils*:** The breast muscles in broiler chickens are classified as skeletal muscles. The smallest unit of skeletal muscle is called a myofibril and a collection of myofibrils forms one muscle fiber (Ridhana, 2018). The results of histological examination of the breast muscles of broiler chickens in each treatment can be seen in Figures 1.

The addition of GAA and betaine as feed additives had a significant effect (P<0.05) on the number of breast muscle myofibrils in broiler chickens. The results of Table 6 show that the average number of breast muscle myofibrils ranged from 147.83-286.33 starting from the lowest in treatment P0 (Commercial Rations) to the highest in treatment P4 (Commercial rations + 0.12% GAA + 0.01% Betaine). This is due to the high feed consumption in the P4 treatment, so that the nutritional needs of broiler chickens are met for the formation of myofibrils in the muscles. Vasilenko *et al*. (2019), added that the number of myofibrils in each muscle fiber varies greatly, resulting in different muscle fiber thicknesses.

Further tests showed that the number of breast muscle myofibrils in broiler chickens in Group I (P0 Vs P1; P2; P3; P4) was significantly different (P<0.05). This is because it contains GAA as an energy source to help muscle growth and production during maintenance. This is in line with the opinion of Mousavi *et al*. (2013), that higher growth and muscle production during the final period of broiler chicken rearing requires more ATP. Furthermore, Boney *et al*. (2019), added that the inclusion of GAA in broiler chicken feed can affect the concentration of phosphocreatine in muscle cells, especially cells in the breast which results in an increase in breast weight which is seen as the main effect of GAA inclusion. Furthermore, in Group II (P1; P3 Vs P2; P4) the results were significantly different (P<0.05) because the addition of betaine in the feed was beneficial for broiler chickens which could increase breast muscle production. Wen *et al*. (2021), reported that among the carcass traits tested, only breast muscle production was increased linearly by betaine.

***Myofibrils Diameter*:** Skeletal muscle fibers are irregularly round and elongated with many cell nuclei attached to the edges of the fiber (Budipitojo *et al*. 2017). The addition of GAA and betaine as feed additives had a significant effect (P<0.05) on the myofibril diameter of breast muscle in broiler chickens. The results of Table 7 show that the average diameter of breast muscle myofibrils ranged from 30.09µm – 54.47µm starting from the lowest in treatment P0 (Commercial Rations) to the highest in treatment P4 (Commercial rations + 0.12% GAA + 0.01% Betaine).

According to Amalo, (2017), the normal diameter of skeletal muscle fibers is generally 10-100 µm. In addition, with increasing age and high levels of activity in livestock, muscle diameter increases. This is in line with the opinion of Bogucka *et al*. (2015) that muscle fiber hypertrophy will increase with age and high levels of activity.

Further tests showed that the myofibril diameter of the breast muscles of broiler chickens in Group I (P0 Vs P1; P2; P3; P4) was significantly different ($P<0.05$). Chen *et al*. (2012) reported that myofibers with a large number of cells have a wider diameter. This is thought to be because supplementation with GAA as a creatine precursor can reduce muscle severity by supplying enough creatine to avoid glycogen depletion and prevent muscle damage (Cordova *et al*. 2018). Cenesiz *et al*. (2020), added that creatine deficiency can cause impaired energy utilization in rapidly growing muscle tissue. Lemme *et al*. (2011), concluded that GAA feed increases muscle creatine concentration, which leads to increased energy metabolism in muscle tissue. Further tests of Group II (P1; P3 Vs P2; P4) on the diameter of breast muscle myofibrils in broiler chickens showed significantly different results ($P<0.05$). This was due to the addition of betaine to the feed which functions to regulate anaerobic muscle glycolysis. This is in accordance with Chen *et al*. (2020), that betaine supplementation can regulate muscle anaerobic glycolysis in broiler chickens.

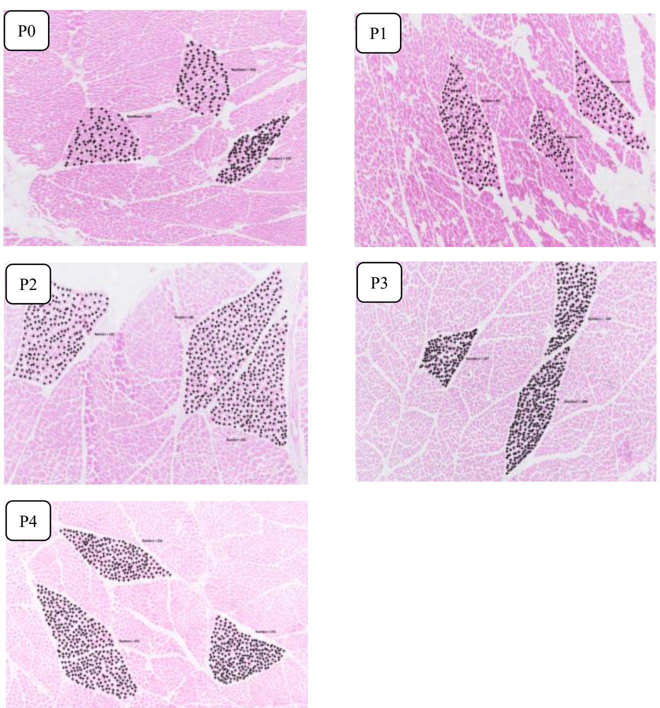

Figure 1. Histology of breast muscle P0 (Commercial Feed as control); P1 (Commercial ration + 0.06 % GAA and no Betaine); P2 (Commercial ration + 0.06 % GAA + 0.01% Betaine); P3 (Commercial ration + 0.12% GAA and no Betaine); and P4 (Commercial ration + 0.12% GAA + 0.01% Betaine).

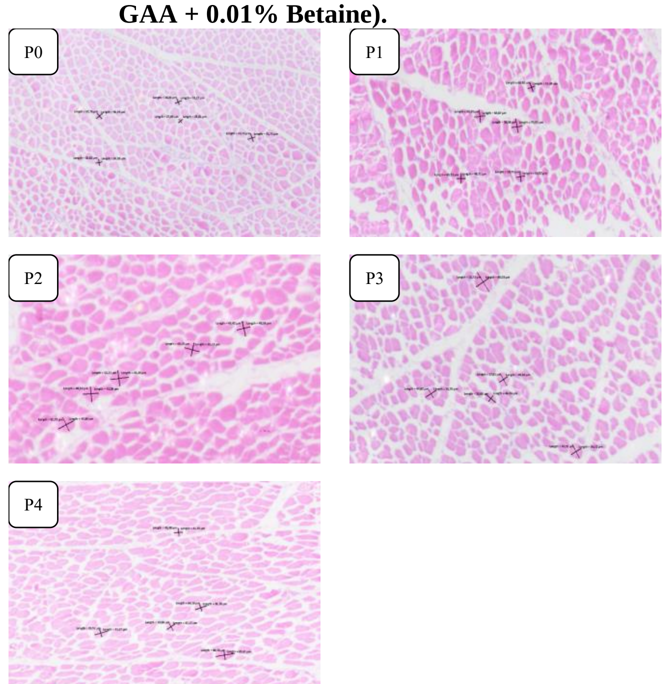

Figure 2. Histology of breast muscle P0 (Commercial Feed as control); P1 (Commercial ration + 0.06 % GAA and no Betaine); P2 (Commercial ration + 0.06 % GAA + 0.01% Betaine); P3 (Commercial ration + 0.12% GAA and no Betaine); and P4 (Commercial ration + 0.12% GAA + 0.01% Betaine).

Apart from that, the addition of betaine to feed also improves growth performance by alleviating the effects of stress in broiler chickens. This is in line with Chand *et al*. (2017) that betaine has been found to significantly improve livestock growth performance and alleviate the detrimental effects of heat stress.

***Conclusion*:** The addition of guanidino acetic acid and betaine as feed additions on performance (feed consumption, body weight gain and FCR), commercial carcasses and histomorphology has a positive influence on broiler chicken. Apart from that, GAA is more effective if methyl donors such as betaine are given in the feed which can increase the number of myofibrils, thereby causing a more effective increase in broiler chicken carcass weight. Suggestions for further research It is necessary to carry out tests with other methyl donors to see the performance function of GAA in broiler chicken feed.

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